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European Patent Office
80298 MUNICH
GERMANY
Tel. +49 (0)89 2399 - 0
Fax +49 (0)89 2399 - 4465

Vossius & Partner
Siebertstrasse 4
81675 München
ALLEMAGNE

Formalities Officer
Name: Yassine / Borger
Tel.: 5762 / 2926
or call:
+31 (0)70 340 45 00

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Reference	Application No./Patent No.
M1335 EP S3	04772194.9 - 2405 / 1666589
Applicant/Proprietor	
TAKARA BIO INC.	

Decision on the request for further processing under Rule 135(3) EPC

The request for further processing received on 09.06.08 has been granted (Art. 121(2) EPC).

☑ ~~The legal consequence notified in the communication~~ dated ..... ~~that the application was deemed to be withdrawn shall~~ not ensue.

☐ The refusal of the application dated ..... shall not ensue.

☐ The legal consequence notified in the communication dated ..... that the particular loss of rights occurred shall not
- ☐ ensue.
- ☐ ensue for the following contracting state(s):

☐ The time limit set in the communication dated ..... is deemed to have been met.

The procedure shall be continued/the particular loss of rights shall not ensue (Art. 121(3) EPC).

For the Examining Division

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Registered letter

VOSSIUS & PARTNER
Patentanwälte Rechtsanwälte

VOSSIUS & PARTNER * POB 86 07 67 * 81634 München * Germany

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EP 04 77 2194.9-2405
Takara Bio Inc.
Our Ref.: M1335 EP S3

Munich, June 6, 2008
SZ/AF

A. REQUEST FOR FURTHER PROCESSING

We herewith request further processing of the present application in accordance with Rule 135 EPC.
The enclosed Form 1010 covers the fee for further processing. The omitted act is completed by providing in the following a response to the Communication pursuant to Article 94(3) EPC dated November 15, 2007.

B. RESPONSE TO COMMUNICATION

Enclosed please find new claims 1 to 17 which should form the basis for further substantive examination.

1. Amendments in the claims

Zur Kasse

1.1 New claim 1 is based on previous claim 1 with the exception that the following amendments have been effected:

(i) Behind the expression "expansion of cytotoxic lymphocytes" the phrase "from a precursor cell

PATENTANWÄLTE
EUROPEAN PATENT ATTORNEYS
EUROPEAN TRADEMARK ATTORNEYS
DR. VOLKER VOSSIUS, Dipl.-Chem. (bis 1992; danach in anderer Kanzlei)
DR. PAUL TAUCHNER, Dipl.-Chem.
DR. DIETER HEUNEMANN, Dipl.-Phys. *
DR. PETER A. RAUH, Dipl.-Chem.
DR. GERHARD HERMANN, Dipl.-Phys.
JOSEF SCHMIDT, Dipl.-Ing.
DR. HANS-RAINER JAENICHEN, Dipl.-Biol.
DR. ALEXA V. UEXKÜLL, M.Sc.
DR. RUDOLF WEINBERGER, Dipl.-Chem.
AXEL STELLBRINK, Dipl.-Ing.
DR. JOACHIM WACHENFELD, Biol.
DR. FRIEDERIKE STOLZENBURG, Dipl.-Biol.
RAINER VIKTOR, Dipl.-Ing.
DR. NATALIA BERRYMAN, Dipl.-Chem.
DR. JÜRGEN MEIER, Dipl.-Biol.
DR. STEFAN FICKERT, Dipl.-Chem.
ARNOLD ASMUSSEN, Dipl.-Ing.(FH)
ELARD SCHENCK ZU SCHWEINSBERG, Dipl.-Ing.
DR. DIRK HARMSEN, Dipl.-Chem.
DR. KATHARINA HAAS, Dipl.-Chem.
DR. CHRISTIAN KILGER, Dipl.-Biol. *
DR. UTE KILGER, Dipl.-Chem. *
DR. ANDREAS KOCH, Dipl.-Biol.

EUROPEAN PATENT ATTORNEYS
DR. RENATE BARTH, Dipl.-Chem.
DR. URSULA ENGLBRECHT, Dipl.-Chem.
DR. PETER EINMAYR, Dipl.-Chem.
DR. WERNER BASTIAN, Dipl.-Biol. **
DR. OLAF MALEK, Dipl.-Biol.
DR. MICHAELA WESSE, Apothekerin
DR. OLIVER SAHR, Dipl.-Phys.
DR. GERHARD WEINZIERL, Dipl.-Biol.
DR. AXEL LEINS, Dipl.-Phys.

RECHTSANWÄLTE
DR. JOHANN PITZ
DR. THURE SCHUBERT
BARBARA GUGGENMOS, Dipl.-Chem.
SIMONE SCHÄFER
JENNIFER CLAYTON-CHEN
DR. NIELS HÖLDER, LL.M.
DR. MATHIAS KLEESPIES, LL.M.
PAUL KRETSCHMAR

CONSULTANTS
HELGA TREMMEL, RECHTSANWÄLTIN
DR. CHRISTIAN GUGERELL, EUROPEAN PATENT ATTORNEY
YOSHIKAZU ISHINO
KIMIO TAKAHASHI

MAIN OFFICE
SIEBERTSTR. 3
81675 MÜNCHEN/GERMANY

POSTAL ADDRESS:
POB 86 07 67
81634 MÜNCHEN/GERMANY

Tel.: +49-(0)89-413 04-0
Fax: +49-(0)89-413 04-111
Fax trademarks: /-400

BERLIN OFFICE *
JOACHIMSTALER STR. 34
10719 BERLIN/GERMANY

Tel.: +49-(0)30-340 609-501
Fax: +49-(0)30-340 609-512

Partnerschaftsregister Amtsgericht München PR 89

BASEL OFFICE (CH) **
NADELBERG 3
4051 BASEL/SWITZERLAND

Tel.: +41-(0)61 560 1490
Fax: +41-(0)61 560 1488

HR Firmennummer CH-270.9.001.271-9

E-Mail:
patents@vossiusandpartner.com
trademarks@vossiusandpartner.com

www.vossiusandpartner.com

which can be formed into the cytotoxic lymphocytes" has been introduced. Support for this amendment can be found on page 21, lines 5 to 13 of the application as filed.

(ii) The feature "4%" has been replaced by "3%". This amendment is supported, e.g., by page 22, last line.

(iii) In the second part of claim 1 starting with "wherein" the reference to substitutions, deletions etc. has been removed.

1.2 New claims 2 to 14 correspond to previous claims 2 to 14.

1.3 New claim 15 corresponds to previous claim 15 with the only exception that the same amendment as set forth in section 1.1(ii) has been effected.

1.4 New claims 16 and 17 correspond to previous claims 16 and 17.

1.5 Previous claims 18 to 20 have been deleted.

2. Article 123(2) EPC

In section 1 of the Communication the Examiner objects to the claims as filed with our submission of August 29, 2007 as violating Article 123(2) EPC, i.e. as adding new subject-matter.

2.1 Claim 1

Claim 1 is objected to with respect to the embodiment referring to SEQ ID NOs: 9 to 20 and 25 or "a polypeptide comprising at least one amino acid sequence having substitution, deletion, insertion or addition of one or the plural number of amino acids in any one of said amino acid sequences and wherein the polypeptide (n) has a function equivalent to that of said polypeptide (m)". The Examiner takes the position that original claims 10 and 12, which had been indicated as providing support for the effected amendment, do not disclose the corresponding feature.

This objection does no longer apply in view of the amendment effected in claim 1 as set forth in section 1.1(iii), supra.

2.2 Claims 1, 6, 13, 14 and 15

Moreover, the Examiner objects to claims 1, 6, 13, 14 and 15 as violating Article 123(2) EPC with respect to the feature "or a mixture thereof".

We respectfully disagree and would like to refer the Examiner's attention to the disclosure content provided on page 12, lines 4 and 5 which says:

> "Each of the above-mentioned fibronectin and a fragment thereof can be used in the present invention alone or in admixture of plural kinds."

This disclosure clearly supports that not only mixtures of fibronectin with fibronectin fragments are considered but also mixtures of different types of fibronectin fragments.

Thus, it is respectfully requested that the objection against clams 1, 6, 13, 14 and 15 is withdrawn.

3. Novelty (Article 54 EPC)

In section 2 of the Communication the Examiner acknowledges novelty of the presently pending claims over D1, D2, D5 and D7. However, the Examiner maintains the novelty objection based on US 5,354,686 (D6).

The Examiner refers on the one side to column 19, line 61 to column 20, line 45 in order to argue that D6 discloses a method of growing cytotoxic lymphocytes such as LAKs in the presence of an extracellular matrix protein such as fibronectin placed on a membrane.

However, the cited passage refers to the following: "The prepared culture of activated lymphocytes -----" (emphasis added). Thus, D6 discloses the step of incubating the activated lymphocytes in the presence of a fibronectin coated solid surface.

In order to distinguish the present invention more clearly from D6, the lymphocytes used for an expansion in the present invention are limited to a precursor cell which can be formed into the cytotxic lymphocytes. By this amendment, it is further clarified that the present invention is different from D6 which relates to activated lymphocytes.

Moreover, we would like to draw the Examiner's attention to the fact that the cited passage does not disclose to culture the cells in a medium containing 0% to 4% serum/plasma. Rather to the contrary, it is stated in column 21, lines 24 to 29 that the cells are most preferably dispersed in LAK medium. However, this medium contains 10% heat inactivated human serum (see column 18, lines 62 and 63). Thus, this passage cited by the Examiner can also for this reason not be detrimental for the claimed subject-matter.

The Examiner goes on to argue that D6 allegedly discloses (in column 31, line 51 to column 32, line 4) that the cells were bound to a proteolytic fragment of fibronectin and the binding was inhibited by 90% by RGDS, the central binding site of fibronectin (column 32, line 65 to column 33, line 37).

However, we respectfully submit that this assessment is not correct. Namely, the passages cited by the Examiner do not deal with the preparation of cytotoxic lymphocytes. The method for preparing the mentioned "EMAT" cells is described in the preceding passage from column 30, line 62 to column 31, line 50. It is described in column 30, lines 63 to 66 that the medium used for the isolation of EMATs involves the use of a complex medium including serum (however without indicating the concentration) and that both collagen IV (bound to the plate) and laminin (in the medium) are used. No mention is made of fibronectin being used in the method for producing the cells.

The passages cited by the Examiner merely relate to morphological studies of the EMATs. Moreover, the cited passage in column 31, line 51 to column 32, line 4 does not disclose, as alleged by the Examiner, that cells were bound to a proteolytic fragment of fibronectin. Said passage only states that "in adhesion assays" the EMAT not only adhere but also spread and form processes on fibronectin. The only other mention of fibronectin (i.e. VLA-5) is in column 31, line 67 to column 32, line 3 where it is stated that another type of T cells, namely Memory T cells, are costimulated via fibronectin (VLA-5) and collagen. However, Memory T cells are different from the EMAT cells the production of which is described in the preceding passage.

Moreover, the passage in column 32, line 65 to column 33, line 37 is not at all related to a method for producing cytotoxic lymphocytes. This passage merely describes (as already evident from the title) morphological aspects of EMATs and reports on the influence certain proteins may have or may not have on T cell activation. However, this passage does not disclose a method involving

expansion of cytotoxic lymphocytes in a medium containing 0% to 3% serum and plasma and a fibronectin fragment.
Thus, we submit that from a careful analysis of D6 it becomes evident that D6 does not disclose a method as claimed in the present application.

Moreover, we also take the position that D6 does not disclose a method which includes an expansion step in a medium containing 0% to 3% serum/plasma and fibronectin (or a fibronectin fragment). Namely, the passage of D6 cited in the Office Action of February 20, 2007 as allegedly disclosing a method for producing cytotoxic lymphocytes in 0-5% serum with fibronectin, i.e. column 5, lines 16 to 61, does not disclose such a method. This passage merely defines the cells and does not disclose any method for their production. The only mention of a serum-free medium is in line 23. However, this mention is in context with a method for determining whether a cell is capable of binding to an extracellular matrix protein. This cannot be regarded as an expansion step.
Moreover, we submit that actually all culture media used in D6 for culturing cells contain serum:

- column 18, lines 19 to 21;
- column 18, lines 52 to 64;
- column 22, lines 17 and 18 mentioning LAK medium which contains 10% heat inactivated human serum;
- column 23, lines 18, 19 and 39;
- column 24, lines 48 to 50; and
- column 30, lines 63 and 64.

Moreover, in the context of the step of binding the cells to a solid surface coated with an extracellular matrix protein, it is actually mentioned that it is preferred to disperse the cells in LAK medium (which contains 10% heat inactivated serum (see column 21, lines 24 to 29)).This is also evident from column 23, lines 27 to 41, describing the preferred protocol.
The only time where an expansion step is mentioned at all in D6 and in which a medium is mentioned, i.e. in column 24, lines 48 to 50, this is carried out with LAK medium which contains 10% heat inactivated human serum.
Thus, we submit that D6 actually does not disclose a method for producing cytotoxic lymphocytes comprising the expansion step as defined in claim 1.
In this context, we would also like to point out that the Examiner's approach which appears to be picking selected pieces of information from D6 out of context

and combining them in a way which can not be derived from D6 is inappropriate. D6 can only be novelty destroying for claim 1 if the claimed subject-matter can directly and unambiguously be derived from D6. However, this requirement is not met if the claimed subject-matter can only be derived from D6 by combining pieces of disclosure which do actually not belong together.

Thus, Applicant submits that the new claims are novel over D6 and respectfully requests that the novelty objection is withdrawn.

4. Inventive step (Article 56 EPC)

4.1 Alleged obviousness of claims 2, 11 to 14 and 17 over D6

In section 3.1 of the Communication the Examiner objects to claims 2, 11 to 14 and 17 as lacking inventive step and chooses as closest prior art D6. It is in particular alleged that D6 already discloses a method for expanding cytotoxic lymphocytes on fibronectin fragments.

We respectfully submit that this assessment is unjustified.
To start with, we believe that the Examiner's statement that D6 has to be regarded as the closest prior art for the subject-matter of claims 2, 11 to 14 and 17 is not correct for the following reasons:
According to the established case law of the Technical Boards of Appeal of the EPO the closest prior art must be a document the teaching of which pursues the same purpose as the invention. Choosing a document as the closest prior art that does not pursue the same purpose as the invention, or in other words, from which the problem according to the invention cannot be derived, is regarded as being based on an inadmissible ex-post-facto analysis (see, e.g., T 435/04, section 4 of the Reasons).
In the present case, the technical problem underlying the present invention is to provide means and methods for producing cytotoxic lymphocytes which can effectively be used in therapy due to a high level of cytotoxicity and which are safe. Thus, as is evident from the present invention, the main purpose is to provide methods which allow, by expanding cytotoxic lymphocytes, preparations of cytotoxic lymphocytes which are more effective than prior art preparations due to a higher number of cytotoxic antigen specific cells and high activity of these cells.

This purpose is not pursued in D6. Rather D6 aims only at isolating T cells which bind to extracellular matrix proteins, in particular γδ T cells (see title and column 4, line 4 to column 5, line 15). Thus, D6 does not pursue the aim of providing an expansion method of cytotoxic lymphocytes in order to prepare more effective preparations of cytotoxic lymphocytes but has a completely different purpose, namely isolating a specific type of T cells, i.e. T cells which bind to extracellular matrix proteins.

Moreover, we would like to again point out that the Examiner's statement that

> "D6 already provides a method for expanding cytotoxic lymphocytes on fibronectin fragments"

is incorrect and untenable (see section 3, supra). As set forth above, D6 does not disclose any expansion step in which a medium as defined in claim 1 is used, let alone by using any fibronectin fragments.

Moreover, even if one would, just for the sake of discussion, accept the Examiner's view that D6 is the closest prior art for claims 2, 11 to 14 and 17, this document cannot render obvious the claimed subject-matter. In particular, D6 does in no way suggest to expand cytotoxic lymphocytes in a medium containing 0% to 3% serum/plasma and fibronectin fragments. As already explained above, when referring to the culturing of cells, D6 always states that the medium contains serum and preferably a medium, i.e. LAK medium is used which contains actually 10% heat inactivated human serum. There is not the slightest hint in D6 that a medium with less serum could be used for expanding cytotoxic lymphocytes.

Thus, it is requested that the above objection for lack of inventive step is withdrawn.

4.2 Alleged obviousness of claims 1 to 15 over D1, D5 and D7

In section 3.2 of the Communication the Examiner takes the position that claims 1 to 15 lack inventive step over D1, D5 and D7. The difference between the claimed subject-matter and D1, D5 or D7 is seen in the feature that 0% to 4% serum/plasma are used rather than 5%, i.e. that the serum concentration is lowered. It is argued that this would have been an obvious measure for the skilled person since it would allegedly have been evident that this would be

beneficial for the patients. Moreover, the Examiner indicates that inventive step could be acknowledged if we could point to advantageous unexpected properties due to the lowering of the serum concentration.

The Applicant submits that this objection does not apply to the new claims.
In new claims 1 and 15, the upper limit of the serum and plasma concentration is limited to 3%. Serum and plasma are very effective ingredients for maintaining and incubating cells. Even if advantages of lowering of serum and plasma concentration were acknowledged, the cited references never suggest any method for lowering the serum and plasma concentration.

It is shown in Example 12 of this application that although the expansion ratio of cells is largely lowered as serum concentration in medium is decreased from 5% to 1% such lowering of the expansion ratio can be remarkably suppressed by the presence of a fibronectin fragment. Also, in Example 55 regarding tests of gradually lowering of serum concentration, it is shown that the reduction of the expansion ratio when the serum concentration is decreased from 5% to 3% or 1% can be suppressed by adding a fibronectin fragment. This effect of compensating for a negative effect due to lowering the amount of serum by a fibronectin fragment is remarkably excellent and unexpected from the cited references so that the present invention is not obvious over the cited references.

4.3 Claims 11 to 14 and 17

The inventive step objections against claims 11 to 14 and 17 raised in sections 3.3 and 3.4 of the Communication do not apply for the same reasons as set forth in sections 4.1 and 4.2, supra.

4.4 Claims 18 to 20

The objection against claims 18 to 20 does no longer apply since these claims have been cancelled.

5. Clarity (Article 84 EPC)

5.1 Claim 1

In section 4.1 of the Communication claim 1 is objected to as lacking clarity because of the feature "at least one amino acid sequence having substitutions, deletions, insertions or additions of one or the plural number of amino acids".

This objection does no longer apply in view of the amendments effected in claim 1.

5.2 Claims 1, 6 and 15

In section 4.2 of the Communication claims 1, 6 and 15 are objected to with respect to the phrase "a fibronectin fragment or a mixture thereof".

The Applicant respectfully disagrees and submits that for the reasons set forth in section 2.2, supra, it is clear what is meant by the objected phrase.

6. Requests

With the above explanations and the proposed amendments to the claims, it is submitted that the Applicant has met the requirements as set forth in the Official Communication.

If, however, the Examining Division does not agree to the above, it is requested that either a further Communication pursuant to Article 94(3) EPC or a summons to attend oral proceedings according to Article 116(1) EPC be issued. If deemed expedient, an informal interview is requested. The undersigned is prepared to discuss minor amendments over the telephone.

Dr. Friederike Stolzenburg
European Patent Attorney

Enclosures
New claims 1 to 17
EPO Form 1010

Claims

1. A method for preparing cytotoxic lymphocytes characterized in that the method comprises the step of carrying out an expansion of cytotoxic lymphocytes from a precursor cell which can be formed into the cytotoxic lymphocytes using a medium containing serum and plasma at a total concentration of 0% by volume or more and less than 3% by volume, in the presence of a fibronectin fragment or a mixture thereof,
wherein the fibronectin fragment is at least one polypeptide selected from the group consisting of polypeptides having any one of the amino acid sequences shown in SEQ ID NOs: 9 to 20 and 25 of Sequence Listing.

2. The method according to claim 1, wherein the method is for preparing cytotoxic lymphocytes which highly express an interleukin-2 receptor as compared to cytotoxic lymphocytes prepared in the absence of fibronectin, a fragment thereof or a mixture thereof.

3. The method according to claim 1, wherein the method is for preparing cytotoxic lymphocytes which contain CD8-positive cell in a higher ratio as compared to cytotoxic lymphocytes prepared in the absence of fibronectin, a fragment thereof or a mixture thereof.

4. The method according to claim 1, wherein the method is for being higher expansion fold as compared to that of a method for preparing cytotoxic lymphocytes in the absence of fibronectin, a fragment thereof or a mixture thereof.

5. The method according to any one of claims 1 to 4, wherein the method is for preparing cytotoxic lymphocytes the cytotoxic activity of which is enhanced or high cytotoxic activity is maintained as compared to a cytotoxic activity of cytotoxic lymphocytes prepared in the absence of fibronectin, a fragment thereof or a mixture thereof.

6. The method according to any one of claims 1 to 5, wherein the fibronectin fragment or a mixture thereof is immobilized on a solid phase.

7. The method according to claim 6, wherein the solid phase is a cell culture equipment or a cell culture carrier.

8. The method according to claim 7, wherein the cell culture equipment is a petri dish, a flask or a bag, and the cell culture carrier is beads, a membrane or a slide glass.

9. The method according to any one of claims 1 to 8, wherein the cytotoxic lymphocytes are lymphokine-activated killer cells.

10. The method according to any one of claims 1 to 9, wherein the fibronectin fragment has a cell adhesion activity and/or a heparin binding activity.

11. The method according to claim 1 which is carried out in a cell culture equipment, wherein the method satisfies the conditions of:
(a) a ratio of the number of cells to a culture area in the cell culture equipment at initiation of culture being 1 cell/cm^2 to 5 X 10^5 cells/cm^2 ; and/or
(b) a concentration of cells in a medium at initiation of culture being 1 cell/mL to 5 X 10^5 cells/mL.

12. The method according to claim 11, wherein the method does not require a step of diluting a cell culture solution.

13. The method according to claim 1, wherein the method comprises carrying out an expansion of cytotoxic lymphocytes in the presence of the fibronectin fragment or a mixture thereof in a cell culture equipment containing a medium, wherein the method comprises at least one step of diluting the cell culture solution, step of exchanging the medium, or step of exchanging the cell culture equipment, and wherein the culture conditions immediately after at least one step of diluting the cell culture solution, step of exchanging the medium, or step of exchanging the cell culture equipment satisfy the conditions of:
(c) a concentration of cells in the cell culture solution being 2 X 10^5

cells/mL to 1 X 10^8 cells/mL; or

(d) a ratio of the number of cells in the cell culture solution to a culture area in the cell culture equipment being 1 X 10^5 cells/cm^2 to 1 X 10^8 cells/cm^2 .

14. The method according to claim 1, wherein the method comprises carrying out an expansion of cytotoxic lymphocytes in the presence of the fibronectin fragment or a mixture thereof in a cell culture equipment containing a medium, wherein the method comprises at least one step of diluting the cell culture solution, step of exchanging the medium, or step of exchanging the cell culture equipment, and wherein a total concentration of serum and plasma in the medium immediately after at least one step of diluting the cell culture solution, step of exchanging the medium, or step of exchanging the cell culture equipment is same as that at initiation of the culture or lowered as compared to that at initiation of the culture.

15. A medium for culturing cytotoxic lymphocytes, characterized in that the medium comprises as an effective ingredient the fibronectin fragment or a mixture thereof, and that a total concentration of serum and plasma in the medium is 0% by volume or more and less than 3% by volume.

16. The method according to any one of claims 1 to 14, further comprising a step of transducing a foreign gene into cytotoxic lymphocytes.

17. The method according to claim 16, wherein the foreign gene is transduced using retrovirus, adenovirus, adeno-associated virus or simian virus.